Proteoglycan metabolism modulation by small molecules for intervertebral disc regeneration

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Methods

Degenerative intervertebral disc (IVD) is characterized by a progressive loss of proteoglycan (PG), the major extracellular matrix component in nucleus pulposus (NP). PG is macromolecules consisting of abundant glycosaminoglycan (GAG) side chains. The large number of carboxyl and sulfate groups in the GAG renders PG a highly hydrophilic nature [1], attracting water molecules and hence providing a high swelling pressure and viscoelastic properties to the IVD to resist compressive loads. Attenuation of proteoglycan production in disc cells is one of the main causes leading to the loss of PG content [2]. Through a high-throughput screen of chemical library, we have previously identified 7 out of 50,240 small compounds that stimulate PG production in porcine chondrocytes and bovine disc cells. In this study we aimed to validate the action of these 7 compounds in human NP cells, with an ultimate goal of developing them into therapeutics for treating IVD degeneration.

Degenerated/Non-degenerated human NP cells

Degenerated Non-degenerated primary NP cells (upper/lower)

Results

Optimization study showed that the reliability and assay window of DMBM assay for the alginate beads were largely affected by the concentration of DMBM and pH. The use of pH=1.5 and 1X DMBM dye provided a reproducible signal at various GAG concentrations compared to the traditional method. In the compounds action test, a general enhancement (10-135 %) of GAG accumulation by the 7 compounds was found in the degenerative human NP cells (Fig. 2). Three of the compounds (CC-3,4 and 5) also significantly stimulated GAG accumulation in non-degenerative NP cells, suggesting they may regulate PG production via pathways common to both non-degenerative and degenerative cells. By comparison, two compounds (CC-6 and 7) mainly exerted their effects on degenerative cells, suggesting they may regulate pathways related to degeneration-induced loss of PG. Interestingly, one of the compounds (CC-1) enhanced GAG accumulation in degenerative NP cells but inhibited it in non-degenerative NP cells.

Discussion

This is the first study of using high-throughput chemical library screening to identify compounds to modulate PG metabolism of degenerative IVD cells. Our in vitro studies indicate that the compounds are effective in promoting GAG production in human NP cells derived from degenerated and non-degenerated IVD. The findings of different patterns of their action imply that degenerative and non-degenerative NP cells may not necessarily share the same PG metabolic pathways. Preliminary analysis of compound structures suggests that the effects of compounds may originate from specific functional group. Moreover, it is possible that the promotion of GAG content by the small molecules may result from an elongation of GAG chains rather than an enrichment of core proteoglycan. Whether the enhancement of sulfated GAG accumulation is actually associated with an increase in PG biosynthesis remains elusive. The action of compounds on the expression of proteoglycan, such as aggrecan, and related enzymes such as ADAMTS and MMPs will be tested in future.

Significance

Our findings may in future facilitate the dissection of regulatory pathways of proteoglycan metabolism in the disc and potentiate the development of new drugs to treat disc degeneration, benefiting millions of low back pain sufferers worldwide.

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References